



Molecular mechanisms of combined heat and drought stress resilience in cereals

Lovely Mae F Lawas¹, Ellen Zuther¹, SV Krishna Jagadish² and Dirk K Hincha¹

Global climate change leads to increased temperatures and decreased precipitation in many parts of the world. The simultaneous occurrence of high temperature and water deficit results in heat stress damage in plants. Cereals provide the majority of calories for human consumption, making this stress scenario particularly threatening for global food security. Several studies in both dicots and cereals indicate that the molecular reactions of plants to combined stresses cannot be predicted from reactions to single stresses. Recent results indicate that the regulation of heat shock proteins and of sugar transport and accumulation in flowers are crucial factors determining resilience of tolerant genotypes to combined heat and drought stress.

Addresses

¹ Max-Planck-Institute of Molecular Plant Physiology, Am Mühlenberg 1, D-14476 Potsdam, Germany

² Department of Agronomy, Kansas State University, Manhattan, KS 66506, USA

Corresponding author:

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Introduction

Global climate models predict a significant increase in intensity and frequency of hot and dry days [1]. Decreased precipitation will lead to more severe drought stress events and high air temperature accompanying drought invariably leads to higher plant tissue temperature. This combination results in heat stress due to insufficient water to meet the evaporative demand [2]. Heat and drought stresses negatively affect the yields of major crops including cereals [3,4], which are staple foods that account for ~60% of the global food energy supply [5]. Despite the frequent occurrence of combined drought and heat stress episodes under field conditions, the majority of studies involving both model and crop

plants have focused on independent heat or drought stress responses [6]. Arguably, imposing combined stress and interpreting the resulting plant responses is highly challenging, due to the biological cross talk between the stress responses [7]. However, the risk that climate change poses to food security has intensified the assessment of the impact of combined stresses on plants and several studies focusing on combined heat and drought stress at the agronomic, physiological, molecular, and metabolic levels have been conducted. [Table 1](#) provides an overview of such studies in cereals over the last 10 years. In this review, we focus on molecular studies investigating combined heat and drought stress in cereals during the reproductive stage and in particular during flowering, which is the most sensitive developmental stage determining grain yield [8]. However, we also present a brief overview of what is known for dicots to provide the necessary context. The review emphasizes the transcriptomic, proteomic and metabolic responses that provide insights into the mechanisms of combined heat and drought stress tolerance and point to the development of potential molecular markers for breeding.

Responses of dicotyledonous plants to combined heat and drought stress

An early molecular study investigating combined heat and drought stress was conducted with tobacco leaves [9] identifying physiological responses such as the closure of stomata, suppression of photosynthesis and increased leaf temperature. In addition, photorespiration was identified as an important metabolic pathway generating reactive oxygen species such as H₂O₂ under combined stress in *Arabidopsis* [10^{••}]. Interestingly, it was also shown that another factor in global climate change, increased atmospheric CO₂ concentrations, reduces the impact of combined heat and drought stress on *Arabidopsis* partially through activation of antioxidant defense mechanisms and reduced photorespiration [10^{••},11]. The latter finding is in agreement with the fact that combined heat and drought stress has a stronger impact on the C₃ plant sunflower than on the C₄ plant maize [12], as C₄ plants have constitutively lower photorespiration.

A transcriptomic analysis in tobacco leaves [9] identified a unique response pattern of gene expression under combined stress conditions. Similar to these results, transcriptomic analysis in poplar [13^{••}] also revealed only a small overlap of genes responsive to combined heat and

Table 1**Reports published during the last 10 years on the effects of combined heat and drought stress in cereals**

Developmental stage	References
Seedling	[33] ^a , [34] ^a , [35] ^b , [36] ^b , [37] ^c , [38] ^d , [39] ^e , [40] ^e , [41] ^e , [42] ^{b,c,e}
Vegetative	[43] ^b
Reproductive	
Pre-flowering	[28] ^a , [44] ^e , [45] ^e , [46] ^e , [47] ^e
Flowering	[31] ^a , [30] ^b , [48] ^b , [49] ^b , [25] ^{**c} , [27] ^c , [50] ^c , [51] ^e , [52] ^e , [53] ^e , [54] ^e
Grain filling	[55] ^a , [44] ^e , [56] ^e , [57] ^e , [58] ^e , [52] ^e , [59] ^e
Whole life cycle	[60] ^{a,e}

^a Barley.^b Maize.^c Rice.^d Sorghum.^e Wheat.

drought stress compared to the single stresses, emphasizing the unique nature of transcriptomic responses to combined stress. Transcriptomic responses involve suppressed photosynthetic gene expression and induced transcripts encoding enzymes catalyzing reactions in glycolysis and the pentose phosphate pathway, pointing to an increased demand for sugars under these conditions. An influence of combined stress on carbohydrate metabolism was also shown in *Arabidopsis* leaves [11], and in lentil [14] and chick pea [15] during reproductive growth and seed filling. In particular, heat and drought sensitive genotypes showed reduced seed size and weight that was caused by a decline of starch and sucrose content and reduced activity of sucrose and starch-synthesizing enzymes.

In contrast to transcriptomic profiles, metabolic profiles of citrus cultivars under combined heat and drought stress were similar to metabolite patterns under heat stress [16]. In addition, the sensitive cultivar showed increased amounts of glycolytic and TCA cycle intermediates, as well as photoprotective and antioxidant secondary metabolites such as flavonoids, while the tolerant cultivar showed much smaller metabolic changes. A transcriptome-metabolome co-analysis in *Arabidopsis thaliana* revealed that combined stress specifically affected genes encoding heat shock proteins (HSPs), enzymes in flavonoid biosynthesis and proteins involved in ribosome biogenesis [17].

A proteomic study on combined heat and drought stress in *A. thaliana* identified 45 proteins specifically accumulating under combined stress conditions. They included enzymes involved in malate metabolism, the Calvin cycle and reactive oxygen species detoxification [18]. In particular, cytosolic ascorbate peroxidase 1 (APX1) was identified as a key player in the response to combined stress, emphasizing the importance of oxidative stress under these conditions as described above. In contrast, a

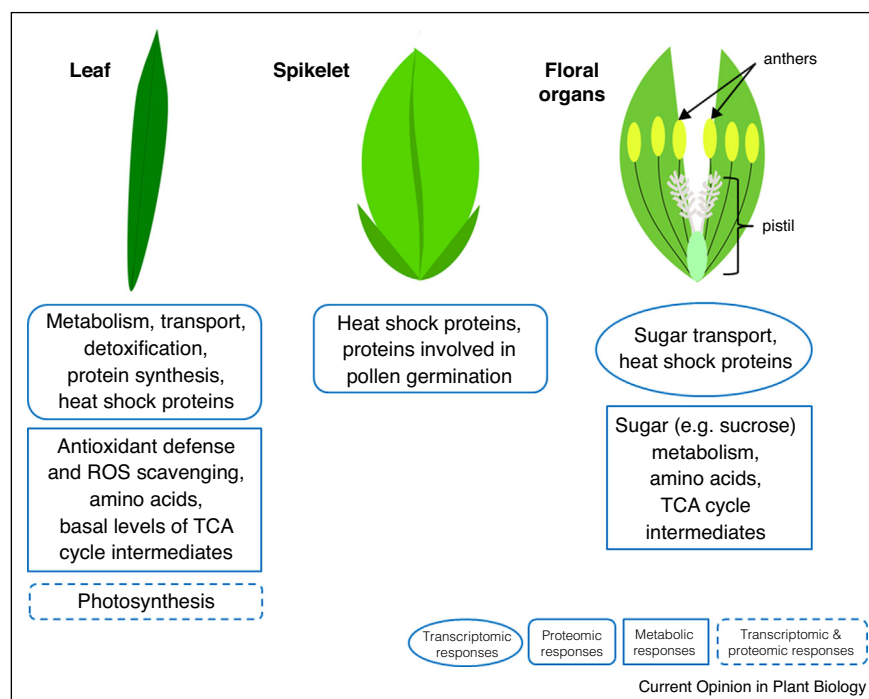
proteomic study in poplar [19] found a decline of antioxidant enzymes under such conditions. However, in both poplar [19] and *Carissa spinarum* [20] several proteins with increased abundance under combined stress were identified as HSPs. A contribution of the co-regulation of several HSPs to the protection of the photosynthetic machinery was postulated for *C. spinarum*. Similarly, many *HSP* genes were found induced in a drought stress study with potato plants grown under field conditions in Germany, indicating that plants were exposed to combined heat and drought stress, although this was not the expressed aim of the study [21]. Further, signal transduction through an ABA-dependent pathway was shown to play a key role in the accumulation of essential proteins during combined heat and drought stress in *Arabidopsis* [22].

Transcriptomic and proteomic responses of cereals to combined heat and drought stress

Similar to dicots, the interaction of heat and drought stresses also induces regulation at the transcriptome and proteome levels in cereals. In both rice and *Brachypodium distachyon*, a grass species that is widely accepted as an experimental model for cereals [23,24], more genes responded uniquely to combined stress than to single stresses [25^{**},26^{**}]. Independent drought stress elicited the least number of treatment-specific differentially expressed genes (DEGs) in *Brachypodium*, in comparison with other single or combined abiotic stresses [26^{**}]. This is in line with the idea that responses to combined stresses cannot be extrapolated from the effects of the individual stresses [6,7]. The DEGs identified in *Brachypodium* as unique under a combination of heat and drought stress were enriched in photosynthetic functions, while DEGs common to single and combined stresses were mostly associated with transport and metabolism of carbohydrates, amino acids, and hormones [26^{**}]. This is in agreement with a study in rice that identified genes associated with combined stress tolerance as related to sugar metabolism [25^{**}]. For instance, the tolerant rice genotype N22 showed higher expression of genes encoding the sugar transporter MST8 and the cell wall invertase INV4 in anthers under combined stress compared to control conditions, while the susceptible genotype Moroberekan showed decreased expression of these genes and induced expression of the gene *Carbon Starved Anthers* (*CSA*).

In contrast to transcripts, no proteins could be identified that showed accumulation exclusively under combined heat and drought stress in rice [27]. Although in rice anthers combined stress elicited more DEGs than heat stress alone [25^{**}], which is consistent with findings at the spikelet protein level [27], more proteins showed increased abundance under drought stress compared with combined stress [27]. Conversely, no significant changes were observed in the leaf proteome of barley subjected to

Figure 1



Graphical summary of molecular and metabolic responses in different organs of cereals to combined heat and drought stress. Organ schemes for leaf, spikelet and floral organs are exemplarily shown for rice.

drought stress, while heat and combined stress resulted in significant changes in the proteome [28[•]]. Differentially accumulated proteins under combined heat and drought are linked to functions related to metabolism, transport, photosynthesis, responses to stress, detoxification and protein synthesis [28[•]]. In addition, HSPs accumulate under combined stress both at the transcript [25^{••},27] and proteome [27,28[•]] levels, showing a conserved protective response between independent heat and drought stresses and their combination in cereals, grasses and dicots. Although a generic response of HSPs to heat stress is well known, the contribution of differential accumulation of specific HSPs to genotypic differences in stress tolerance remains to be investigated.

Collectively, these studies show the dynamic regulation between the transcriptome and proteome under stress conditions. In addition, there are obviously species-specific mechanisms of coping with different stresses and stress combinations and molecular stress reactions also differ between organs such as leaves and flowers and even between different tissues within organs, such as anthers and pistils (Figure 1).

Metabolic responses of cereals to combined heat and drought stress

With the recent advances in metabolomics, a number of studies have employed this technology to assess plant

responses to abiotic stresses [29], including combined heat and drought stress in cereals. Li *et al.* [25^{••}] studied the metabolic changes induced by this stress combination in rice anthers, as well as in unpollinated and pollinated pistils. These floral tissues had distinct metabolite profiles under both control and stress conditions. The levels of amino acids and sugars were significantly changed under combined stress, with changes differing between the tolerant genotype N22 and the susceptible genotype Moroberekan. Nine metabolites that may confer protection against combined heat and drought stress were identified, three of which showed higher levels in the tolerant genotype already under control conditions. These nine metabolites include metabolites involved in the TCA cycle and in sugar metabolism, such as succinate, isocitrate, sucrose and *myo*-inositol. Significantly, combined stress resulted in a significant decrease in sucrose levels in the anthers of Moroberekan.

On the other hand, simultaneous heat and drought stress resulted in only a few significantly affected metabolites in the leaves of field-grown maize plants, of which most were shared responses under the single heat and drought stresses [30]. Moreover, the responses to combined stress could be predicted from the sum of the effects of the individual stresses, in contrast to transcriptomic responses as discussed above. The levels of TCA cycle intermediates (e.g. fumarate, malate, succinate) under control

conditions were suggested in this study to be potential metabolic markers to predict grain yield under combined stress, while the photorespiratory amino acids Gly and Ser, *myo*-inositol and raffinose family oligosaccharides were proposed to be important for yield under drought. In barley flag leaves, more metabolites were accumulated under combined stress than under drought. These included amino acids such as Pro, Gln and Gly, and antioxidant tocopherols [31]. The genotypes that were included in this study differed in their drought stress tolerance and showed differential changes in the levels of several metabolites under drought. However, changes in metabolite levels were similar in all genotypes under combined stress.

Similar to the conclusions drawn above from the transcriptomic and proteomic studies, the results of the metabolomic studies indicate the presence of responses that are conserved among species, while others may be more species-specific. It is also obvious that different plant organs, such as leaves and flower tissues, differ in both their metabolite composition under control conditions and in their metabolic reactions to stress (Figure 1). However, due to the limited number of metabolomic studies in this area, it is presently not possible to draw any more detailed conclusions. In particular, it will be interesting to see whether the sugar starvation phenotype observed in the anthers of a susceptible rice genotype [25**] can also be observed in other cereal species.

Future directions

Due to the limited knowledge of molecular mechanisms underlying combined heat and drought tolerance in cereals, a number of intriguing questions need to be answered to understand plant tolerance under these conditions. This will facilitate efficient breeding and developing crops that are resilient towards such challenging climatic conditions (see Box 1). Obviously, some of these questions are also of interest to the wider field of plant stress tolerance research beyond the focus of this review.

Box 1 Outstanding questions

- How different are the molecular signatures of tissues or organs in response to combined heat and drought stress?
- How and to what extent can results from controlled climate chamber experiments be extrapolated to plants grown in the field?
- Can transcriptomic, proteomic and metabolomic approaches be used to identify molecular markers to speed up breeding for combined heat and drought tolerance in cereals?
- What is the quantitative contribution of different HSPs to combined heat and drought stress tolerance and what is their mode of action?
- Can sugar starvation in anthers under combined heat and drought stress be alleviated by increasing appropriate transporter activity?

As outlined above, many reactions of cereals to combined heat and drought stress are tissue-specific or organ-specific (Figure 1). This makes comparisons between studies using different organs (e.g. flowers versus leaves) virtually impossible. We believe that future studies should strongly concentrate on understanding tolerance at the flowering and early grain filling stages to be as relevant to the final grain yield as possible. In addition, direct comparisons between field and climate chamber grown plants will be necessary to evaluate the extent to which findings from controlled conditions can be extrapolated to the agronomically relevant field situation.

Two major reactions of plants to combined heat and drought stress stand out from current studies: the massive induction of HSPs and the sugar starvation phenotype in anthers (Figure 1). Transgenic approaches will be necessary to evaluate whether the introduction of specific HSPs, or of sugar transporters will be sufficient to alleviate stress symptoms to increase yield under combined heat and drought stress. Finally, studies with larger and genetically diverse populations differing in stress tolerance will be necessary to conduct genome-wide association studies to identify genomic regions and candidate genes for combined stress tolerance. Investigating such populations over multiple years in multiple locations will allow using metabolite and transcript profiling to identify markers for efficient marker-assisted breeding, as recently implemented in potato [32].

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